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SOME FURTHER EXPERIMENTS ON SELF-FERTILIZATION IN CIONA.

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My previous experiments with *Ciona intestinalis* had shown that, by adding chloroform, or alcohol, or ammonia to the sea water, self-fertilization could sometimes be effected, although in this species self-fertilization does not usually take place in sea water. The experiments did not show why under normal conditions self-fertilization does not occur. Other results appeared to indicate that the eggs offer a resistance of some kind to the entrance of the spermatozoa of the same individual and that the lack of fertilization is due to the failure of the spermatozoa to enter, and not due to their failing to stimulate the eggs after entering. An analysis of the conditions led me to the conclusion that the inability of the spermatozoa to penetrate eggs of the same individual could not depend on differences in size of the micropyle of the egg that might be correllated, supposedly, with the size of the spermatozoa of the same individual ; also that the failure was not due to the lack of some exciting substance present in eggs of other individuals. I hazarded the guess that the resistance offered by the egg to the entrance of its "own" sperm might be due to some substance contained in the egg, or in its membranes, that brings the spermatozoa of the same individual to rest, and I pointed out that this view could be tested by the following experiment. If the eggs of an individual (A) were removed and allowed to stand in sea water, and if then this water were poured off and the spermatozoa of the same individual (A) were added to the water, they should be so affected that they would fail to fertilize the eggs of another individual (B). I should have made the *proviso*, as subsequent events have shown, that this result would follow, provided the substance that prevents self-fertilization is soluble in sea water. This experiment I have carried out during the past summer in a variety of ways, but have found that the anticipated results did not follow, from which I am led to conclude that the phenomenon is not due to a substance that is soluble.

The failure of this experiment to give positive results showed me that I must start once more at the very beginning, and test more thoroughly the assumption that the sperm of one individual, if in good condition, is capable of fertilizing equally well the eggs of all other individuals; for the difficulty of explaining the results becomes immensely greater if this condition holds absolutely. If it does hold it would mean that the conditions present in an individual are not found in any degree in any other individual. The principal results of this paper deal, therefore, mainly with this question, although, at the same time, I have described some other experiments which gave negative results, because, I think, the results, negative though they be, will be of value in determining the direction of further experiments.

My work was carried out at the marine laboratory of the University of California, situated at present at Coronado Beach, California. I am under many obligations to the university for the privilege of working at the station, and especially to the director, Professor William E. Ritter, and to the resident naturalist, Mr. B. M. Davis. It gives me great pleasure to acknowledge my appreciation of the many courtesies extended to me during my sojourn at the station. Professor Ritter informs me that the species of *Ciona* at Coronado Beach appears to be identical with *Ciona intestinalis* of Europe.

Experiments in Cross-fertilization.

The eggs of an individual were removed and distributed in six dishes, A–A. Similarly the eggs of another individual were taken out and distributed in six dishes, B–B; and, so on, for four other individuals. See Table I. The sperm of A was then removed from the vas deferens and added to A, B, C, D, E, F. In the table the sperm is indicated by the small letters, a–f, used as exponents. The sperm from each of the other five individuals was used in the same way. Thus all the eggs were crossed with all the different kinds of sperm, and self-fertilization was also tried in each case. The self-fertilized lots form a diagonal line across the tables. In only a single case out of the many hundreds of eggs mixed with their own sperm did fertilization occur. It is needless, perhaps, to add that separate scissors, pipettes, etc., were used for each individual. In each experiment there were thirty-six fertilizations made, of which thirty were crosses, and six self-fertilizations. In the eight experiments that I carried out there were therefore 240 cross experiments, which, if not a very extensive series, yet ought to suffice to show the main points. The percentages given in the following tables indicate the proportionate number of eggs in each set that segmented; the estimated number are approximations only, and not, in all cases, the results of exact counts.

Table I.

$\mathbf{A}^a$	A^b 90	A^c 80	A^d 10	A^e 0	A^f 0
B^a 0	$\mathbf{B}^b$	B^c 3	B^d 0	B^e 0	B^f 0
C^a 100	C^b 80	$\mathbf{C}^c$	C^d 100	C^e 75	C^f 0
D^a 90	D^b 75	D^c 80	$\mathbf{D}^d$	D^e 0	D^f 0
E^a 20	E^b 25	E^c 0	E^d 0	$\mathbf{E}^e$	E^f 0
F^a 0	F^b 0	F^c 0	F^d 0	F^e 0	$\mathbf{F}^f$

An analysis of the results shown by this table leads to the following conclusions. The A-eggs were in good condition (or more briefly were "good"), since with "good" sperm 90 per cent. of them were fertilized in one case. The B-eggs were poor, the C-eggs excellent, the D-eggs very good, the E and F-eggs were poor. The a-sperm was excellent, as seen in C^a; the b, c, d, e-sperm were also excellent; but the f-sperm was very poor, since not a single egg in any of the sets was fertilized by it. From the results of this table it looks as though good sperm would always fertilize good eggs of any other individual, but there are even here three significant exceptions to this statement. The d-sperm gave poor results with A-eggs (A^d), although other experiments show that both the sperm and the eggs of these two individuals were good. The other cases are those of A^e and D^e, in which the e-sperm failed completely with the A and with the D-eggs, although with the C-eggs it gave 75 per cent. of segmenting eggs.

There was also another point that I wished to test by means of these experiments, namely, whether there is any correspondence between normal and abnormal development of the eggs and the proportion of the eggs that are fertilized. It will be recalled in this connection that Castle found that those cases of *Ciona intestinalis* in which self-fertilization occurs development is abnormal. On the contrary, I found that self-fertilized eggs often give rise to normal embryos. I wished, therefore, to see whether in cross-fertilized eggs there is any relation between the number fertilized and the condition of the embryos. In the following table the kinds of embryos that developed from the eggs given in the preceding table are recorded. The abnormal records generally indicate tadpoles that were bent, or crooked, or otherwise abnormal in shape.

TABLE I.—*Continued.*

$\mathbf{A}^a$	A^b Abn	A^c Abn	A^d Abn	A^e 0	A^f 0
B^a 0	$\mathbf{B}^b$	B^c Abn	B^d 0	B^e 0	B^f 0
C^a Abn	C^b Abn	$\mathbf{C}^c$	C^d Abn	C^e Abn	C^f
D^a Abn	D^b Abn	D^c Abn	$\mathbf{D}^d$	D^e 0	D^f 0
E^a Abn	E^b Abn	E^c	E^d	$\mathbf{E}^e$	E^f 0
F^a 0	F^b 0	F^c 0	F^d 0	F^e 0	$\mathbf{F}^f$ 0

The table shows that all of the tadpoles were abnormal, despite the fact that a large percentage of the eggs was fertilized in several cases. The next table gives the results of a second experiment.

TABLE II.

$\mathbf{A}^a$	A^b 10^b	A^c 40	A^d 50	A^e 0	A^f 40
B^a 30	$\mathbf{B}^b$	B^c 90	B^d 80	B^e 1	B^f 25
C^a 90	C^b 95	$\mathbf{C}^c$	C^d 99	C^e 100	C^f 100
D^a 90	D^b 90	D^c 80	$\mathbf{D}^d$	D^e 1	D^f 99
E^a 0	E^b 40	E^c 25	E^d 50	$\mathbf{E}^e$	E^f 0
F^a 1	F^b 40	F^c 80	F^d 15	F^e 0	$\mathbf{F}^f$

The A and the E-eggs were only moderately good, the B, C, D, and F-eggs were very good. The sperm in all the sets gave very good results with at least some of the eggs. The exceptional cases found in this series were as follows. The a-sperm did poorly with B, very poorly with F, and gave nothing with E, although with C and with D 90 per cent. of the eggs were fertilized. The b-sperm also gave varying results, although the extremes were not so marked ; and this holds to even a less degree with the c-sperm. The c-sperm gave exceptionally good results with the F-eggs. There is nothing calling for comment in the behavior of the d-sperm; while the e-sperm was surprisingly deficient with the B and the D-eggs, although excellent with C. The f-sperm gave excellent results with the C and the D-eggs, but was less good with B. The later development of the eggs in this series was not observed.

Table III.

A^a	A^b 80	A^c 80	A^d 1	A^e 0	A^f 0
B^a 100	**B^b**	B^c 100	B^d 10	B^e 2	B^f 1
C^a 100	C^b 100	**C^c**	C^d 20	C^e 25	C^f 1
D^a 100	D^b 100	D^c 50	**D^d**	D^e 5	D^f 10 (1 in 10)
E^a 20	E^b 10	E^c 10	E^d 2	**E^e**	E^f 0
F^a 20	F^b 70	F^c 15	F^d 1	F^e 0	**F^f**

The A, B, C, D-eggs appear to have been excellent; the E-eggs poor, and the F-eggs probably good. The a and the b-sperm appear to have been good, and the latter, in the case of F, gave unexpectedly good results. The c-sperm was also good, but gave with D only 50 per cent. of segmenting eggs. The d, e, f- sperm was poor.

The embryos (tadpoles) were examined after 24 hours with the results recorded in the following table:

Table III.—*Continued.*

A^a	A^b Abn.	A^c Abn.	A^d Abn.	A^e One abn.	A^f 0
B^a Abn.	**B^b**	B^c Nearly norm.	B^d Abn.	B^e Abn.	B^f One norm.
C^a Norm.	C^b Norm.	**C^c**	C^d Most abn. One norm.	C^e Most norm.	C^f One norm.
D^a Most abn.	D^b Most abn.	D^c Abn.	**D^d**	D^e Abn.	D^f Abn.
E^a Abn.	E^b Abn.	E^c Abn.	E^d	**E^e**	E^f Abn.
F^a Abn.	F^b Abn.	F^c Abn.	F^d 0	F^e 0	**F^f**

Although A^b and A^c gave 80 per cent. of cases of fertilization they produced only abnormal tadpoles. B^a gave also only abnormal tadpoles, while B^c gave nearly normal tadpoles. Although only one egg in B^f was fertilized it produced a normal tadpole. The C-eggs gave even more curious results. The C^a and C^b-eggs gave exceptionally normal and active tadpoles; the C^d-eggs were abnormal (excepting for one individual); most of the C^e were normal, while the one egg fertilized in C^f was normal. The D^a and D^b-eggs gave very abnormal tadpoles, but some of them were normal, while D^c and D^e and D^f were abnormal.

All of the E and the F-eggs gave abnormal tadpoles. These results show very clearly that there is no necessary relation between the percentage of eggs fertilized and the normal or abnormal development that takes place, although in general good eggs are more likely to produce normal tadpoles, and poor eggs abnormal ones.

TABLE IV.

$\mathbf{A}^a$	A^b 15	A^c —	A^d —	A^e —	A^f —
B^a 30	$\mathbf{B}^b$	B^c 35	B^d 5	B^e 0	B^f 0
C^a 5	C^b 0	$\mathbf{C}^c$ 1 in 10	C^d 0	C^e 0	C^f 0
D^a 20	D^b 10	D^c 3	$\mathbf{D}^d$	D^e 8	D^f 0
E^a 100	E^b 100	E^c 95	E^d 100	$\mathbf{E}^e$	E^f 0
F^a 75	F^b 1 in 10	F^c 4 in 26	F^d 1 in 50	F^e 0	$\mathbf{F}^f$

In this series the A-eggs were not distributed through a mistake except in two cases. The B and the D-eggs were poor; the C-eggs very poor; the E-eggs were excellent; and the F-eggs seem to have been good. The a-sperm gave the best results, yet failed to give high percentages with the poorer eggs; and the b-sperm behaved similarly, but did less well with F. The c and the d-sperm were excellent with the e-eggs only, while the e and the f-sperm were poor in all cases, showing, in the case of the E-eggs, which were excellent, that the latter could not fertilize eggs as good as these.

TABLE V.

$\mathbf{A}^a$	A^b 0	A^c 0	A^d 0	A^e 0	A^f 100
B^a 100	$\mathbf{B}^b$	B^c 5	B^d 0	B^e 0	B^f 80
C^a 30	C^b 10	$\mathbf{C}^c$	C^d 0	C^e 0	C^f 80
D^a 0	D^b 2	D^c 0	$\mathbf{D}^d$	D^e 0	D^f 14
E^a 5	E^b 25	E^c 5	E^d 0	$\mathbf{E}^e$	E^f 30
F^a 75	F^b 20	F^c 2	F^d 0	F^e 0	$\mathbf{F}^f$

This experiment gave some curious results. The A-eggs must have been excellent to judge by A^f, yet gave no results in other cases. The B-eggs must have been excellent, but gave good results only with the a and the f-sperm. The C-eggs gave similar results, but few eggs segmented with the a-sperm. The D-eggs must have been poor giving only 14 per cent. with the f-sperm. The E-eggs also gave poor results,[1] and the F-eggs

[1] The E-eggs were polyspermic.

were well fertilized only with the a-sperm. The development of the eggs as shown by the following table gave some unexpected results when considered in the light of the number fertilized :

Table V.—*Continued.*

$\mathbf{A}^a$	A^b Norm.	A^c Abn.	A^d o	A^e o	A^f Norm.
B^a Abn.	$\mathbf{B}^b$	B^c Norm.	B^d o	B^e o	B^f Norm.
C^a Norm.	C^b Abn.	$\mathbf{C}^c$	C^d o	C^e o	C^f Abn.
D^a ?	D^b Abn.	D^c o	$\mathbf{D}^d$	D^e o	D^f Abn.
E^a Abn.	E^b Abn.	E^c Norm.	E^d o	$\mathbf{E}^e$	E^f Abn.
F^a Norm.	F^b Norm.	F^c o	F^d o	F^e o	$\mathbf{F}^f$

The B^a-tadpoles were abnormal although 100 per cent. of the eggs were fertilized ; while the B^c-tadpoles were exceptionally normal. Although the c-sperm was very poor, the B^c and the E^c-tadpoles were exceptionally active and normal. The E^f-tadpoles were only somewhat abnormal, although the eggs appear to have been poor. The F^a and the F^b tadpoles were excellent, although only 20 per cent. of the eggs divided in the latter case.

Table VI.

$\mathbf{A}^a$	A^b o	A^c o	A^d 20	A^e o	A^f o
B^a 100	$\mathbf{B}^b$	B^c 25	B^d 75	B^e 65	B^f 95
C^a 90	C^b 90	$\mathbf{C}^c$	C^d 90	C^e 90	C^f 50
D^a o	D^b 2	D^c o	$\mathbf{D}^d$	D^e 20	D^f 20
E^a 20	E^b 95	E^c 40	E^d 100	$\mathbf{E}^e$	E^f 80
F^a 1	F^b 95	F^c 30	F^d 100	F^e 40	$\mathbf{F}^f$

The most striking results shown by this table are that while the F-eggs were excellent and the a-sperm also, yet F^a gave only one per cent. of segmenting eggs. The e and f-sperm gave much better results with D-eggs, than did any other sperm with these eggs, although some of the other sperm appears to have been excellent.

Table VII.

$\mathbf{A}^a$	A^b o	A^c o	A^d 8	A^e o	A^f o
B^a 15	$\mathbf{B}^b$	B^c 5	B^d 5	B^e o	B^f o
C^a 70	C^b 80	$\mathbf{C}^c$	C^d 95	C^e 80	C^f 20
D^a 12	D^b 1	D^c 8	$\mathbf{D}^d$	D^e 6	D^f o
E^a 50	E^b 70	E^c 50	E^d 95	$\mathbf{E}^e$	E^f o
F^a 80	F^b 60	F^c 30	F^d 95	F^e 75	$\mathbf{F}^f$

In this table also there are several cases where the sperm was less capable with some eggs than with others, even when other cases showed that the eggs were equally good. The differences are not so striking as in some of the other series.

TABLE VIII.

A^a	A^b 20	A^c 85	A^d 75	A^e 0	A^f 35
B^a 0	**B^b**	B^c 20	B^d 25	B^e 0	B^f 0
C^a 0	C^b 0	**C^c**	C^d 8	C^e 0	C^f 0
D^a 8	D^b 50	D^c 90	**D^e**	D^e 0	D^f 7
E^a 0	E^b 8	E^c 80	E^d 50	**E^e**	E^f 0
F^a 0	F^b 3	F^c 8	F^d 15	F^e 0	**F^f**

With the exception of c- and of d-sperm the sperm appears to have been very poor in this series. The B, C and F-eggs were also poor. It is noticeable that the results appear to depend in this case more on the condition of the sperm than of the eggs.

In my previous paper five tables,[1] similar to these, were given for the *Ciona* found at Woods Hole. The incompatability was less marked, although some cases, apparently of this sort, were found. In general, however, the eggs crossed much more readily than did the California form. Since the individuals at Woods Hole were collected over a wider area than were those at Coronado Beach, all of which grew on the same float, the chance is greater that they may have had a different parentage. Whether the closer descent may account for the greater incompatability in the Coronado individuals can not be stated, but the facts are suggestive.

DISCUSSION OF EXPERIMENTS.

The most obvious question that suggests itself in those cases where few eggs were fertilized is whether if more sperm was added more eggs would segment. In answer to this I should point out that an approximately equivalent amount of the same solution containing sperm was added in each vertical series. Another experiment made to test this point showed that the amount added was many times greater than that necessary to fertilize the eggs when a good combination is made. Thus 3, 6,

[1] A few errors in my former tables may be corrected here. Thus in all the tables A^e appears twice, and E^a also. In experiment XIV. the first A^e gave 0 per cent., the second 100. This is due no doubt to the failure to add sperm to the first lot, which might easily occur. This is apparently also the case in experiment XV. In experiment XVI. the first E^a gave only 5 per cent., the last 70. This difference I cannot now account for. B^c in this table should be B^d, and C should be C^e.

12 and 24 drops of a solution containing the sperm was added to equivalent numbers of eggs, and gave 90, 90, 90, 100 per cent. of eggs fertilized. In another case 3, 6, 12 and 24 drops gave 6, 50, 90, 90 per cent. In the latter case three drops were not enough; six not quite enough; but 12 and 24 sufficed. Now since in all the other experiments more than 24 drops were used the outcome must depend in only a small degree on the quantity of sperm, and the results represent, therefore, approximately the proportion of eggs and sperm capable of uniting.

The tables show that the sperm is generally "at fault," when fertilization does not occur, the eggs being in nearly all cases capable of fertilization when good sperm is used, although, as pointed out, some striking exceptions occur. Thus poor sperm will sometimes fertilize more eggs of one set than of another, but the better the sperm the more eggs it will fertilize as a rule.

It is clear from the tables that it is erroneous to suppose that the sperm will fertilize equally the eggs of all other individuals. All degrees of sterility are met with in cases where other experiments with the same sperm fully succeed. Therefore the problem is not so sharply defined as appeared to be the case before these experiments were made.

Experiments to Determine the Nature of the Influences Preventing Self-Fertilization.

As stated above these experiments were undertaken in the hope of finding out whether there is a soluble substance in the eggs or body tissues of an individual that so affects its own sperm that it is rendered incapable of fertilizing its own eggs. If this is the case it would seem probable that the sperm might be affected by extracts of its "own" tissues, so that it might no longer be capable of cross-fertilization, if eggs of another individual were added to the extract containing the sperm.

The experiments to test this are not so simple as may appear on first thought, since check experiments must be carried out in order to see whether the same action may not result when extracts of the body of another individual are used. In fact it would have been very easy to make a serious blunder had not this precaution been taken.

The converse experiment should also be carried out at the same time; namely, that in which the sperm of A is put into the extract of B in order to see if it might not be made active so that it would then fertilize its own eggs. In my previous paper I have described some experiments of this kind. As a check to this the a-sperm should be put into the B-extract and then later the B-eggs added to show whether the extract itself may not interfere with the fertilization. The complete experiment is indicated in the following table:

A-sperm.	B-extract.	Add A-eggs	(1)
		Add B-eggs	(2)
	A-extract.	Add A-eggs	(3)
		Add B-eggs	(4)
B-sperm.	B-extract.	Add A-eggs	(5)
		Add B-eggs	(6)
	A-extract.	Add A-eggs	(7)
		Add B-eggs	(8)

Experiment 1.—In one experiment in which an extract of the ovary of the individuals was used none of the eggs segmented showing that even the extract of another individual prevents the cross-fertilization from taking place. A test experiment with these same eggs and sperm, brought together in sea water, showed that A-eggs with B-sperm gave only 20 per cent. of cases of segmentation, and B-eggs with A-sperm gave no cases of fertilization. It is clear, therefore, that the eggs and sperm were not in very good condition. When self-fertilized in sea-water neither the A-eggs or the B-eggs segmented.

Experiment 2.—In another experiment the eggs and the ovary of one individual (A) were soaked in sea water for one hour. The same was done with the eggs and the ovary of another individual (B). The sperm of A was added to the A-extract and the B-sperm to the B-extract for half an hour. Then the B-eggs were placed with the A-sperm that had been in the A-extract, and the A-eggs with the B-extract and B-sperm. None of the eggs divided. In this same experiment the B-sperm was put into the extract of the ovary of A, and later the A-eggs were added; similarly for the A-sperm. No eggs were cross-fertilized, showing as in the preceding case that the extract itself had interfered with cross-fertilization (provided the eggs were good which was not tested).

Experiment 3. — In this experiment the eggs (instead of the sperm) were soaked for a short time in the extract of the ovàry of another individual in order to see if they might not acquire the power to become fertilized by the sperm if this were then added.

The A-eggs were soaked in the extract of the ovary of B about 15 minutes. The A-sperm was added to sea water, and left for 5, 10 and 15 minutes. Then the A-eggs were added to the A-sperm solutions. No eggs segmented, but neither did the eggs in the check experiment in sea water, showing that the eggs or the sperm were poor, or else incompatible. In the check experiment (B-eggs in extract of A-ovary), however, 100 per cent. of the B-eggs were fertilized by the A-sperm. This also happened in the cross in sea water. Here the A-extract did not prevent the B-eggs from being cross-fertilized by A-sperm. The solution must have been sufficiently dilute for fertilization to take place.

Experiment 4. — In this case the mantle was removed, and the body wall slightly cut so that the heart protruded, which was cut open and the blood collected. The eggs of another individual were then put into the blood. There is here a possible source of contamination, since some of the follicles of the testis may be broken, and allow some of the sperm to get into the blood. The results of one experiment of this sort were as follows :

			In Blood.	Diluted Later.
(1)	A-eggs	A-blood, B-sperm	0	40 %
(2)	B-eggs	A-blood, A-sperm	0	70
(3)	A-eggs	B-blood, B-sperm	0	0
(4)	B-eggs	B-blood, A-sperm	0	0

The check crosses carried out in sea water gave for the A-eggs (B-sperm) 90 per cent. of cases of segmentation, and for the B-eggs (A-sperm) 50 per cent. The first two combinations (1) and (2) of the table, gave no results in the blood itself, but when the blood was diluted they gave nearly the same percentages as in sea water. In the second cases the dilution was probably insufficient. Thus it is shown that the undiluted blood interferes with the fertilization, but on the other hand the diluted blood of the same individual does not affect the sperm so that it fails to cross-fertilize.

Experiment 5. — The eggs were put into the blood of another individual for twenty minutes, and then removed to sea water, and their own sperm added. No self-fertilization followed, although direct cross-fertilization gave 70 per cent. for the B-eggs, and 100 per cent. for the A-eggs, showing that both eggs and and sperm were good.

Experiment 6. — In order to test whether the blood itself may not sometimes become contaminated with its own sperm, one portion of the blood of A was diluted with sea water, and B-eggs were then put into it. In one case a few eggs segmented, showing that contamination may occur. Heating the blood would, of course, kill the sperm, but this would open the experiment to the objection that the composition of the blood might be so affected that the postulated soluble substance is destroyed. Since most of the experiments gave negative results the contamination of the blood, if it occurred, would not vitiate such results ; but if the results had been positive this possibility would havehad to be carefully reckoned with.

Effects of Shaking.

It seems not improbable that the covering of test-cells might be the immediate cause of the lack of power to self-fertilize. Therefore I tried the effect of shaking them off, and adding sperm of the same individual to the denuded eggs.

Experiment 7. — In this case only one egg out of 200 segmented, while 400 of the same eggs unshaken showed no segmentation.

Experiment 8. — In another case about 20 per cent. of the shaken eggs segmented, but some of those that segmented had still some of the follicle cells attached. In another instance 8 out of a total of 40, or 20 per cent., segmented.

These results show unmistakeably that shaking increases the percentage of self-fertilizations that take place, but whether on account of the removal of the follicle cells, or from some other change induced in the eggs is not certain.

Experiment 9. — In this case none of the shaken eggs segmented ; although the crossed eggs gave 50 per cent. in one case, and 100 in another showing that the eggs and the sperm were good.

Effect of Follicle Water.

If some substance in the follicle cells excites cross-fertilization, then it seemed likely that the water in which the eggs had been violently shaken, so that the follicle cells broke apart, might act on the sperm or eggs of another individual, and cause self-fertilization. This was tried in a few cases.

Experiment 10. — In one case five per cent. of the eggs divided; in three cases there were no divisions; in another case 70 per cent. divided, but went only into the two- or four-cell stages, which seemed to show that something unusual had taken place, and that the results were not due to the follicle water. There is, in fact, a source of contamination in this experiment that may fully account for all the cases observed. In removing the eggs from the oviduct some of the sperm from the vas deferens may be accidently squeezed out and become mixed with the eggs, and remaining in the follicle water fertilize the other eggs. The shaking might injure the sperm, but those that escaped might still suffice. The peculiar segmentation in the case in which a considerable number of eggs segmented may have been due, in fact, to the injury to the sperm. Separate experiments must be undertaken to test this possibility. The negative results in these cases are probably the more significant.

Effects of Standing.

Experiment 11. — The relative age of the eggs might, it seemed to me, be a factor in the result; also by standing in sea water the substance that prevents self-fertilization might be washed out. To test these possibilities, eggs were removed and kept for eight hours in one case in sea water and then self-fertilized with their own sperm that had remained in the animal. No results followed. That the eggs are not injured by standing in sea water was shown by their power to become cross-fertilized even after 24 hours.

Effects of Bursting the Eggs.

Experiment 12. — By compressing the eggs between a cover slip and a slide they can be burst in some cases. I attempted then to self-fertilize the egg-fragments, etc. Many of the eggs that had been broken were self-fertilized, indicating that the resistance to self-fertilization is due to something in the membranes surrounding the eggs. The experiments need to be repeated on a larger scale, but even if placed beyond doubt, the explanation of what there is in the membranes that prevents self-fertilization remains still to be determined.

Extract of the Sperm.

Experiment 13. — Wishing to see if an extract of the sperm of one individual would cause the sperm of another individual to fertilize its own eggs, the following experiment was made. The sperm of three individuals was dried in the sun on a piece of glass; the residue, including the salt, was scratched off, and as much tap water was added as there had been sea water before. Sperm and eggs of another individual were then added to the solution, but no results followed.

Diluting the Water.

Experiment 14. — In order to determine whether dilution of the sea water might not bring about self-fertilization, the following experiment was made: One, two, three, four or five parts of tap water were added respectively to one hundred parts of sea water. In all of these solutions cross fertilizations took place, but in no case did self-fertilization occur.

Experiment 15. — In this case the eggs were first crossed and then put into sea water, diluted as follows: 40, 50, 60, 70 parts of tap water were added to 100 parts each of sea water. In the first and second solutions about half of the eggs segmented into several cells; in the third solution few eggs segmented, and none in the fourth, although they became polynuclear. The eggs of three individuals were put, with their own sperm, into the same solutions, but gave no results.

Summary.

1. It is not true that the sperm of a given individual will fertilize equally well the eggs of all other individuals.

2. The eggs appear to be in good condition much oftener than the sperm, but even good eggs cannot be fertilized *equally well* by good sperm of all other individuals.

3. In the case of poor sperm the discrimination shown between the eggs of different individuals is apparently more marked than in the case where the sperm is unusually good.

4. The results are not due to different amounts of sperm used in different cases, because the same, or nearly the same amount was used in each series. Other experiments showed that the amount of sperm used was many times greater than that sufficient to fertilize all the eggs present. On the other hand by using a larger amount of "poor" sperm the percentage of cases of cross-fertilization could probably be increased.

5. There is no definite relation between the number of eggs that are cross-fertilized and the normal or abnormal condition of the tadpoles. Previous experiments had also shown that perfectly normal tadpoles may be produced by self-fertilization in the few cases in which this takes place naturally or is induced.

6. If a strong extract of the ovary of an individual (A) is made, and the sperm of A (first made active in sea water) is added, and if then the eggs of B are also put in, they may not be fertilized if the solution is very strong, but if it is diluted fertilization may take place. Thus the A-sperm is not brought to rest by an A-extract, except in so far as the solution is too strong to allow any fertilization, as is shown when A-sperm is put into B-extract and B-eggs added — no fertilization taking place in the strong solution, but occurring if dilution is subsequently brought about.

7. If A-sperm is put into an extract of the ovary of B, and then A-eggs are added no fertilization occurs, showing that the extract of another individual does not excite the sperm to self-fertilization.

8. If the *eggs* of an individual (A) are placed in an extract of the ovary of B, then returned to sea-water and A-sperm added, self-fertilization does not occur. The extract of B does not effect the A-egg so that they will self-fertilize.

9. The blood and extracts of the body tissues of another individual give similar negative results.

10. Shaking the eggs, so that the follicle cells are removed, favors self-fertilization.

11. Placing the eggs and sperm of one individual in the "follicle-water" (obtained by shaking) of another individual gave distinctly increasing percentages of apparent self-fertilization, but the results are probably due to contamination.

12. Extracts of the sperm of other individuals did not, in the few cases tried, induce self-fertilization.

13. Dilution of the sea water does not facilitate self-fertilization, although cross-fertilization may still occur in water considerably diluted.

14. Eggs after standing for 24 hours in sea water are not fertilized by their own sperm.

15. Bursting the eggs, so that the membrane is ruptured or the egg is set free, allows self-fertilization to occur in a large number of cases, showing that the lack of self-fertilization of the unbroken egg is probably due to a resistance found in the surface of the egg, in the membrane, in the follicle cells or in their secretions.

Hypothetical and Speculative.

The immunity of the eggs of *Ciona* to its "own" sperm invites a comparison with cases of immunity to infectious diseases. The more so since Landsteiner and Metschnikoff have found that when, for example, spermatozoa of the ox are injected into the guinea-pig they remain active for some time, but if the injections of the ox sperm are continued at proper intervals the guinea-pig makes a spermatoxin that quickly brings to rest all later-injected spermatozoa. If something similar occurs in *Ciona* the reaction is much more delicate than any heretofore discovered, and would open a wide field for future investigation.

Without wishing at present to press this point too far it seems to me of sufficient weight to warrant calling attention to some of the implications in the case of *Ciona*.

The presence of eggs and sperm in the same individual might appear to give an opportunity for a reaction similar to that just described. If the eggs, and also the body tissue of the animal, were supposed to make a counter substance that would bring the spermatozoa to rest, we might account for the lack of self-fertilization in this way. Further, the proximity of the sperm-duct and the oviduct in *Ciona* suggests that the eggs might all the more easily be stimulated to form the substance.

There is also another possibility, viz., that a substance may be secreted by the sperm-duct that keeps the mature spermatozoa at rest, and that this substance may affect the near-lying eggs, so that they also may acquire this property. Unless, however, this influence on the eggs were of a peculiarly individual sort, it would prevent all fertilization, cross- as well as self-fertilization, which is not the case.

There remains still another possibility which may better account for the conditions. It may be that the result does not depend on a reaction between the sperm and the tissues or eggs of the same individual, but to a similarity depending on the common descent of the sperm and the eggs. Owing to their close similarity of composition, the activity of the sperm on coming in contact with its "own" egg-membranes may be decreased, so that the spermatozoön can not force its way into the egg. This point of view offers certain advantages over the others mentioned, especially when extended to some other animals. Thus in the bee, the spermatozoa are stored up in the receptaculum of the female, but the fertilization of the eggs of the female is not thereby prevented. In this case the spermatozoa and the eggs have arisen from separate individuals, and, hence, fertilization is possible, despite the fact that the sperm is stored in the body of the female that contains the eggs. In some hermaphroditic animals and plants self-fertilization occurs, but this is not a fatal objection to the hypothesis, because, although in these cases also a condition similar to that in *Ciona* may be supposed to exist, it may not be sufficiently strong to prevent the sperm from entering the eggs — the activity of the sperm being greater, or the reaction being less marked. The fact that in some of these cases self-fertilization takes place less readily than cross-fertilization is distinctly in favor of the present point of view.

The greater activity induced in the sperm of *Ciona* by ether and other exciting substances may make them sufficiently active to break through the barrier around their "own" eggs, and having once entered the egg, the stimulus caused by the nucleus or by the centrosome may cause the development to proceed.

If the spermatozoa are brought to rest by substances of some sort on the surface of the egg, or in its membranes, these may be a part of the living substance, and not set free in the sea water. Hence the failure to detect such bodies in the water or extracts under the crude condition of the experiments.

Violently shaking the eggs may remove entirely, or in part, the protecting covering, so that self-fertilization may more often take place.

If eggs are shaken in sea water, so that pieces of the follicle cells are set free, these pieces would bring to rest all of their "own" sperm that came into contact with them, but the number of spermatozoa in the water might be too great for all of them to be caught, and those that remained might suffice to fertilize the eggs of another individual if they were then added to the water. Thus we can see why in the experiments cross-fertilization was not prevented when the sperm was first added to its own follicle water.

The fact that cross-fertilization was so often incomplete in the San Diego form, and so much more frequent in the form from Woods Hole, suggests that the difference may be due to the closer relationship of the former group of individuals. The Woods Hole individuals were collected over a wider area, and other conditions at this place make it more probable that these individuals may have had a separate descent. The close resemblance, in fact, between the infertility of *Ciona* when self-fertilized, and the infertility of closely inbred forms is apparent, and the same explanation may apply to both cases.

The immunity of the eggs of *Ciona* to sperm of the same individual suggests a comparison with the cases of immunity to infectious diseases, but it is evident that the two cases can not be identical, for the antitoxin of disease is supposed to combine with the toxin of the poison, and thus inhibit its action. It would be absurd to suppose that there are bodies in the egg large enough to lock up, as it were, the spermatozoa; but on the other hand it is not impossible that substances may exist that quiet the activity of the spermatozoa, and that these substances are a part of, or the immediate product of, the protoplasm of the individual, and are not produced by a reaction between the body tissues (or the eggs) and the spermatozoa of the same individual, as in the case mentioned above when the sperm of one animal is injected into another.